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Peripatopsidae (Onychophora) from New Zealand - observations on selected morphs of the '*Peripatoides novaezealandiae*-complex' in culture: morphological and reproductive aspects

by

Birgit Pripnow and **Hilke Ruhberg**

(Zoologisches Institut und Zoologisches Museum, Abt. NT1, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany; birgit@pripnow.de)

ABSTRACT

This study was undertaken in order to obtain more data on the life history of Onychophora under laboratory conditions, and to help distinguish selected morphs of the '*Peripatoides novaezealandiae*-complex'.

Morphs of the '*P. novaezealandiae*-complex' were observed in culture, and on the basis of live specimens, a preliminary key was developed for the identification of six different morpho types. These can be distinguished by the following characters: colour and shape of the genital aperture, converging or non-converging ventral anal papillae, presence or absence of two orange-coloured 'ocular' papillae, and the shape of the nephropore on the 4th and the 5th legs.

Data from adults of six morphs and from 41 offspring of four morphs were recorded and illustrated in order to facilitate future taxonomic research. Supplementary information on juveniles was due to observations of 27 individuals from two additional morphs. The females, probably iteroparous seasonal breeders, gave birth after 17 to 24 months in captivity. They appeared to be able to defer release of embryos or to fertilise eggs with stored sperm. Morphs could be distinguished by the different duration of their reproductive periods.

Based on changes in pigmentation and on the brightness of the integument in new born animals, three stages were distinguished: stage I, glistening neonates with varying degrees of pigmentation; stage II, juveniles with stronger pigmentation, but without lustre; stage III, fully pigmented juveniles. The degree of pigmentation is found to correlate with the morpho type. Moreover, the shape and the morphology of the anal cone in morphs aged two and five months can be used to distinguish gender.

INTRODUCTION

Literature on the taxonomy of the Onychophora is, for the main part, incomplete (Ruhberg 1992). In addition, museum material is often old, type material is lost or incorrectly labelled, and there is no taxonomic standardisation in the literature. Onychophora show remarkably low morphological diversity, so every observation on live animals is significant and of great importance in improving taxonomic information.

The ovoviviparous *Peripatoides novaezealandiae* (Hutton, 1876) belongs to the family Peripatopsidae. At present, five species from two genera in this family are known from New Zealand and about 20 to 30 more await description. Hence, the onychophoran fauna of New Zealand is potentially more extensive than is evident from literature (Gleeson & Ruhberg 2000), and the Peripatopsidae from this region are currently under revision (Ruhberg & Gleeson, in prep.).

Peripatoides novaezealandiae s. str. was first described from a locality near Wellington (New Zealand). It has long since been regarded as the most widespread species of Onychophora in New Zealand, but it has recently been established that *P. novaezealandiae* is a complex of several different species (Ruhberg 1999). Consequently, attempts are underway at present to investigate the taxonomy by comparing data from physiology, morphology, histology, and genetics.

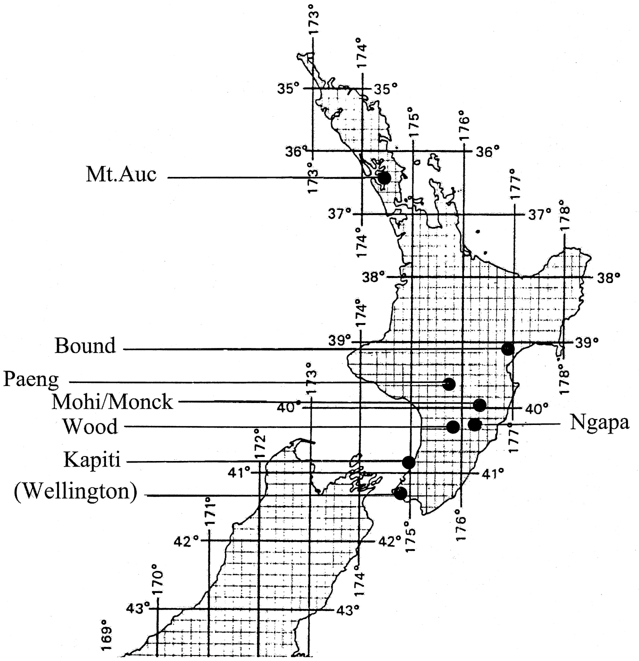

Fig. 1: New Zealand localities of observed Peripatopsidae: the abbreviations are for Mount Auckland, Boundary Stream, Paengaroa Mataroa, Mohi Bush, Monckton, Woodville Gorge, Kapiti Island, and Ngapaerera. Part of 'base-map': Modified after Crosby *et al.* (1976).

With few exceptions it has not been possible to create artificial conditions enabling onychophorans to complete their reproductive cycle in culture. Onychophora (e.g. *Peripatopsis moseleyi*, *P. sedgwicki*, *P. balfouri* and *P. capensis*) usually stop producing offspring after nine months in captivity (Manton 1938).

For this study animals of six different morphs of the '*P. novaezealandiae*-complex' were examined under laboratory conditions in Hamburg, Germany. Juveniles from two additional locations, Mount Auckland and Ngapaerera, were also investigated. Locations shown in Figure 1 are Boundary Stream, Paengaroa Mataroa, Mohi Bush, Monckton, Woodville Gorge, Kapiti Island, Mount Auckland and Ngapaerera.

This paper attempts to use morphological and some reproductive aspects of living morphs of the '*P. novaezealandiae*-complex' in culture, in order to enhance differentiation between new taxa. This is the first 'captive study' of this kind on any species of the New Zealand Peripatopsidae. In future it will be necessary to compare the data obtained here with more studies on the same and on other morphs in captivity, as well as with additional data from natural populations.

MATERIAL AND METHODS

The conditions under which the observations reported here were undertaken were difficult. There were few specimens of each morph to investigate, so no statistical analysis was possible. Furthermore, most onychophorans are hard to rear in captivity because they are extremely sensitive. Also, it was impossible to collect new specimens during

TABLE 1.
List of numbers of adult females and males observed from each locality.

Location	Number of males	Number of females	Total number of animals
Boundary Stream	2	1	3
Kapiti Island	1	2	3
Mohi Bush	1	1	2
Monckton	7	3	10
Paengaroa Mataroa	-	4	4
Woodville Gorge	2	1	3

the study period, so priority was given to studying live animals rather than preserved specimens.

Twenty five live adults (collected by Tutt, Gleeson, Hall and Ruhberg from six locations in New Zealand's North Island between February 1997 and November 2000) were kept in special containers with perforated lids, organised with respect to their locations (Fig. 1; Table 1). There were between two and five adults in every box. They lived under moist tissue layers and on moist turf that was pressed tightly to hinder them from hiding under it to facilitate better observations. Unfortunately no males from Paengaroa Mataroa and, after the death of a single female, no females from Woodville Gorge were available.

Because of their sensitivity, disturbance of the animals was kept to a minimum during the four months in which their morphological characteristics were investigated. Only a cold-light source (KL 750 SCHOTT) was used during the observations. This produces intensive, yet heat-free illumination in order to put minimum stress on the onychophorans. Morphological features of these onychophorans were examined using a stereo-microscope (OLYMPUS SZH10). One male from Monckton was killed and morphological features were examined. Results were documented by sketches (using a drawing tube: magnification factor 1.25 x), photomicrographs taken with a stereo-microscope, and by scanning electron microscopy (LEO 1525 Gemini). Photomicrographs (using a Leica R4) were also taken of some embryos dissected from a dead female from Monckton, as well as of some premature hatchlings.

Altogether 68 juveniles of six different morphs (from Boundary Stream, Paengaroa Mataroa, Monckton, Woodville Gorge, Mount Auckland, and Ngapaerera) were monitored until the newborn were five months old. In particular the pigmentation and brightness of the integument were investigated. The shape of the genital pore and anal cone was documented for juveniles of the morphs from Boundary Stream, Paengaroa Mataroa, Monckton, and Woodville Gorge.

RESULTS

Morphological characteristics of adults

The base colour of all observed Peripatopsidae was a dark bluish grey. However, the different morphs had varying numbers and patterns of orange papillae. All animals examined had a bulge of orange-coloured papillae situated in a latero-ventral position relative to the eyes (Fig. 2). This was considered to be a good characteristic for morphs

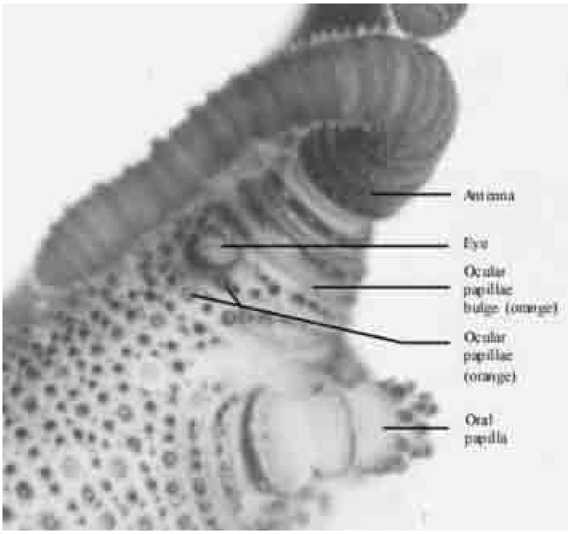

Fig. 2. Head of Monckton male, lateral view (20 x).

of the '*P. novaezealandiae*-complex'. Furthermore, animals from Mohi Bush, Monckton and Woodville Gorge had two orange-coloured 'ocular papillae' (Fig. 2), whilst animals from the other three locations lacked these.

The animals investigated had 15 pairs of legs, with three complete sole pads on the ventral part of each leg, and an additional fragmented one, proximal to the others (Fig. 3). Three distal papillae were present on every foot. The shape of the nephropore was hard to differentiate because the animals were moving, but it was apparent that its size varied between animals.

The male genital pores were circular in shape and either orange-coloured or brownish grey with an orange frame (Fig. 4). The female genital pores were circular, swollen, and lipped, and again either orange-coloured or brownish grey. Interestingly, the lateral, orange-coloured anal papillae of the males from all locations studied here were converging (Fig. 13), except for the anal papillae of Woodville Gorge males (Fig. 14).

On the basis of these observations, the six morphs proved to be morphologically distinguishable. A preliminary key was developed that could be extended to include other morphs and could possibly be used for field work.

Preliminary key for six morphs of the '*Peripatoides novaezealandiae*-complex'

1 Two orange-coloured 'ocular papillae' present in a latero-ventral position relative to the eyes 2
– Two 'ocular papillae' absent 4

2 Lateral anal papillae of males converging 3
– Papillae not converging .... Woodville Gorge

3 Female genital pore orange, swollen and lipped, rather long. Nephropore subdivides 3rd sole pad of foot in half or more .... Monckton

– Female genital pore brown-grey, swollen and lipped, circular. Nephropore subdivides 3rd sole pad of foot in half or less.. Mohi Bush

4 Nephropore subdivides 3rd sole pad of foot more than halfway 5

– Nephropore subdivides 3rd sole pad halfway (at 5th pair of legs further than half)………… .... Paengaroa Mataroa

5 Male genital pore orange coloured, circular .... Boundary Stream

– Male genital pore brown-grey, orange framed, circular .... Kapiti Island

Data from births

In this study, a total of six females each produced offspring during the observation time. This is remarkable considering the time they had been kept in captivity prior to this study, namely 17 to 24 months, and also considering the relatively short observation period.

Some onychophorans accidentally gave birth to premature hatchlings that did not survive; for example, a female from Kapiti Island had 22 premature hatchlings.

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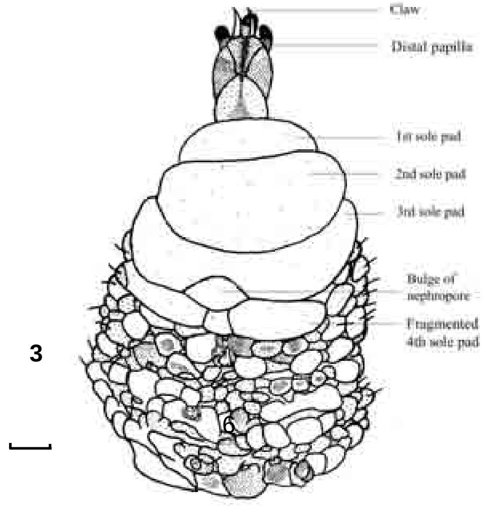
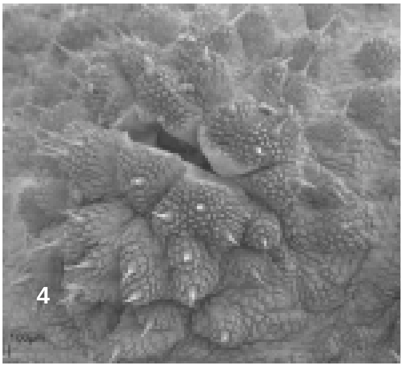
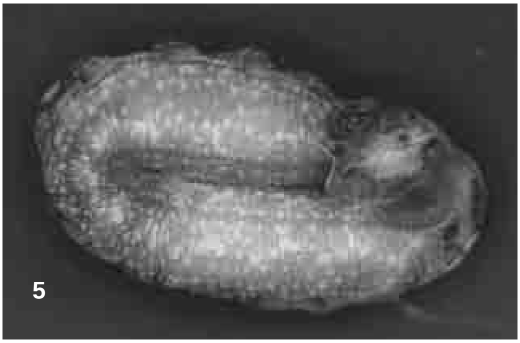






Figs 3–5. Diagram, micrograph and photograph of selected morphs. 3. Schematic sketch of Mohi Bush male: fifth leg, ventral view (Scale bar = 163 µm). 4. SEM micrograph of genital pore: Monckton male (Fig. rotated approx. 45˚). 5. Monckton: premature hatchling (25 x).

TABLE 2.

Number of juveniles born during this study from females of different locations.

Location	**Boundary Stream**	**Kapiti Island**	**Mohi Bush**	**Monckton**	**Paengaroa Mataroa**	**Woodville Gorge**	**Mount Auckland**	**Ngapaerera**
Number of females	1	–	–	1	1	1	1	1
Number of juveniles	13	–	–	3	8	17	12	15
Timing of birth periods	Feb.–Mar.	–	–	Apr.–May	Jul.–Sept.	Feb.–Mar.	May–Jun.	May–Jun.
Duration of birth periods	1 month	–	–	1 month	3 months	1 month	1 month	1 month

Most of the premature hatchlings were not morphologically differentiated. Exceptions were: a blue-whitish premature onychophoran from Monckton (Fig. 5); a premature hatchling from Boundary Stream (Fig. 6), whose slime gland had slipped through a wound in the skin of the animal so that the oral tube—already filled with glue—could be seen; and another premature animal from Boundary Stream, on which an exuvium was seen.

Early stages of embryos from *P. novaezealandiae s. str.* and from Monckton looked superficially identical, but later stages varied: there was a longitudinal and a semicircular ridge seen on the head, as well as a longitudinal ridge on the body of *P. novaezealandiae s. str.* embryos, whilst embryos from Monckton lacked these.

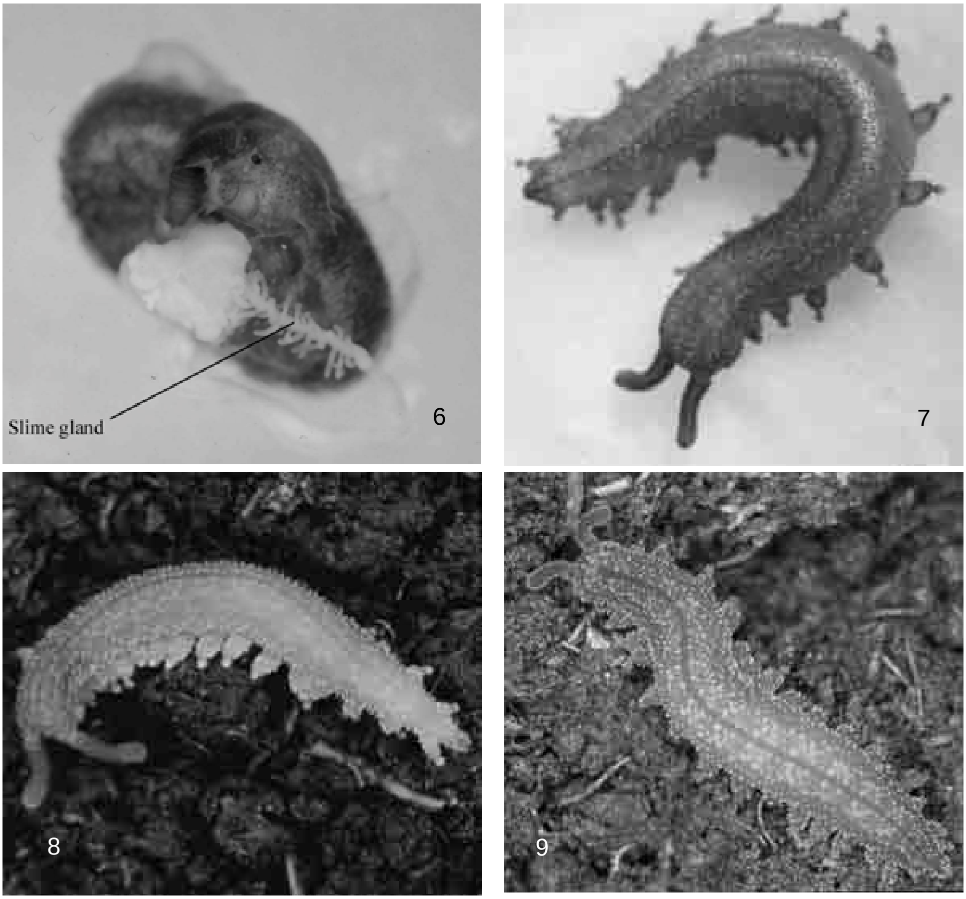
Six females gave birth to a total of 68 juveniles during the short investigation period. The reproductive period of the observed morphs during which all juveniles of each female were born was usually one month long, but took place in different months for the different morphs (Table 2). In contrast, the reproductive period of the Paengaroa Mataroa morph was longer, namely three months.

Pigmentation and brightness of the integument in new born animals

All the observed morph offspring were born with pigments, in contrast to the white-coloured neonates of *P. novaezealandiae s. str.* (Tutt 1997; Brosius 1998). The consistent base colour of the morph neonates was a dark bluish grey, except for newborn from Boundary Stream, which had a light white-purple tint, and neonates from Mount Auckland, which had a light brownish beige or brownish blue coloration. All newborn from the different morphs had varying numbers and patterns of orange papillae. Literary sources confirm that coloration of the integument of juveniles becomes stronger during the first two weeks after birth (Ruhberg 1981).

In this study three stages of pigmentation and coloration of the integument were distinguished in offspring:

- **Stage A**: neonates shortly after birth: the integument appears wet and always has a silvery sheen (Fig. 7), except for the integument of Mount Auckland juveniles, which has no such sheen. The integument shows varying degrees of pigmentation. The base colour is mainly dark grey or white-purple. The sensory spines of the papillae are exposed like needles (Fig. 8).
- **Stage B**: the integument is more strongly pigmented, but without lustre. The sensory



Figs 6–9. Selected hatchlings and juveniles. 6. Boundary Stream: premature hatchling with slime gland (25 x). 7. Juvenile from Ngapaerera: stage A (7 x). 8. Juvenile from Boundary Stream: stage A (7 x). 9. Juvenile from Monckton: stage C (10 x).

spines of the papillae are still exposed to some extent. Stage B of the integument was developed at the latest nine days after birth. The change to stage C is hard to determine.

- **Stage C**: the integument is fully pigmented and without lustre. The papillae resemble those of adults (Fig. 9).

The time to reach the fully developed stage C differed markedly between the morphs, which might be one method of distinguishing them. Stage C was reached after less than 25 days after birth by juveniles from Monckton and Woodville Gorge, up to 50 days after birth by juveniles from Boundary Stream, and more than 50 days after birth by juveniles from the other three locations, Ngapaerera, Paengaroa Mataroa, and Mount Auckland.

Anal cone and genital pore in two and five month old juveniles

There have been no previous reports describing the shape of anal cones and genital openings or the development of these structures in juveniles. Examination of these

TABLE 3.
Gender determination of juvenile morphs from different locations.

Locations	Number of juveniles observed for gender	Gender determination after 2 months		Gender determination after 5 months	
		females	males	females	males
Boundary Stream	13	–	–	13	–
Monckton	1	–	1	–	
Paengaroa Mataroa	5	–	5	–	–
Woodville Gorge	17	5	10	2	–

features in live animals is difficult because of the risk that they might die as a consequence of disturbance. Therefore, several offspring from the same location were examined for very brief periods using a stereo-microscope. Only juveniles that survived five months after birth were included in this investigation (Table 3).

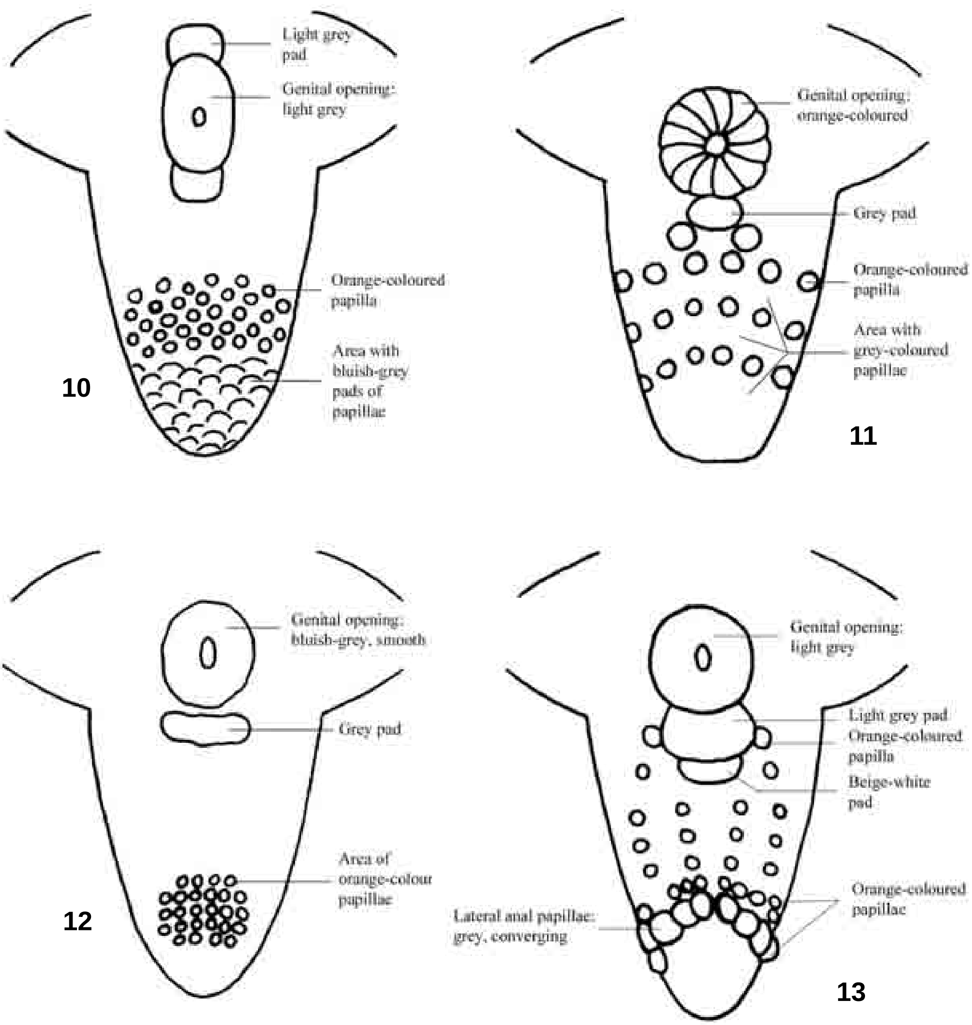
Two months after birth gender determination was impossible for onychophorans from Boundary Stream (for details of ventral anal cone, see Fig. 10). In addition, gender was not determined for two juveniles from Woodville Gorge after two months (Fig. 12), but they were found to be females after five months. However, gender determination was possible for all other observed juveniles two months after birth. Nonetheless, their genital pores were more differentiated five months after birth than earlier.

An extraordinary sex ratio was that of morphs from Boundary Stream and Paengaroa Mataroa. In total, the female from Boundary Stream produced no males but 13 females (Fig. 11), and the female from Paengaroa Mataroa produced no females but five males (Fig. 13). The female from Woodville Gorge produced altogether 10 male (Fig. 14) and seven female (Fig. 15) offspring. The only surviving offspring from Monckton was male.

Genital openings of undifferentiated Boundary Stream (Fig. 10) and Woodville Gorge (Fig. 12) juveniles (two months old) were either light grey (Boundary Stream) or bluish grey (Woodville Gorge). They were framed by either two grey pads (Boundary Stream) or one pad (Woodville Gorge). Towards the posterior end of the body a zone of orange-coloured papillae could be distinguished in juveniles of both of these morphs (Boundary Stream and Woodville Gorge).

Five month old females from Boundary Stream (Fig. 11) and Woodville Gorge (Fig. 15) had orange genital openings, and areas of orange and grey papillae. The genital pore was framed by either one (Boundary Stream) or two (Woodville Gorge) grey pads. Genital pores of five month old Paengaroa Mataroa (Fig. 13) and Woodville Gorge males (Fig. 14) were light grey. They had two pads caudally as well as a zone of orange papillae, in rows in the case of Paengaroa Mataroa. As in male adults of this morph, the lateral orange anal papillae were either converging (Paengaroa Mataroa) (Fig. 13) or not converging (Woodville Gorge) (Fig. 14).

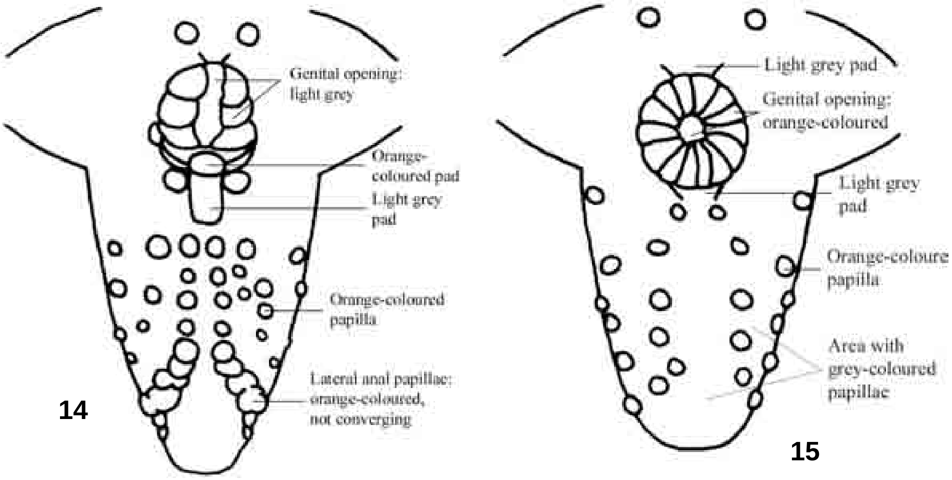
During the investigation of juveniles with the stereo-microscope many of them deposited drops of urine at the nephropore of the fourth and fifth pair of legs. This was also observed during weight recordings.


Light grey pad
Genital opening: light grey
Orange-coloured papilla
Area with bluish-grey pads of papillae
10
Genital opening: orange-coloured
Grey pad
Orange-coloured papilla
Area with grey-coloured papillae
11
Genital opening: bluish-grey, smooth
Grey pad
Area of orange-colour papillae
12
Genital opening: light grey
Light grey pad
Orange-coloured papilla
Beige-white pad
Orange-coloured papillae
Lateral anal papillae: grey, converging
13

Figs 10–13. Sketches of posterior ventral body surface. 10. Juvenile from Boundary stream, 2 months, genital area and papillae of anal cone still undifferntiated (30 x). 11. Juvenile from Boundary Stream, 5 months, female (30 x). 12. Juvenile from Woodville Gorge, 2 months, genital area and papillae of anal cone still undifferentiated (30 x). 13. Juvenile from Paengora Mataroa, 2 months, male (30 x).

DISCUSSION

The observed morphs were morphologically differentiated by the following significant characters: absence or presence of two orange-coloured ‘ocular papillae’; converging or non-converging anal papillae of males; form and colour of male and female genital pore; position and size of the nephropore. However, the morphological characters were mainly observed on live, moving animals. As a consequence the results should be verified by more observations and using preserved material.


Genital opening:
light grey
Orange-
coloured pad
Light grey
pad
Orange-coloured
papilla
Lateral anal papillae:
orange-coloured,
not converging
14
Light grey pad
Genital opening:
orange-coloured
Light grey
pad
Orange-coloure
papilla
Area with
grey-coloured
papillae
15

Figs 14–15. Sketches of posterior ventral body surface. 14. Juvenile from Woodville Gorge, 5 months, male (30 x). 15. Juvenile from Woodville Gorge, 5 months, female (30 x).

Reproductive periods varied between the ovoviviparous morphs. Hence, parturition seems to be dependant on either season or, more probably, on certain environmental factors or population differences. Some of Ruhberg's unpublished records on reproductive periods of morphs from Kapiti Island and Paengaroa Mataroa also suggest an absence of seasonal dependence. Fertilisation of the females studied here may have taken place either in nature or in the laboratory, but the fertilisation of the female from Paengaroa Mataroa must have taken place in New Zealand at least two years ago, because no males from this location were cultured. This demonstrates that females must be able to either defer release of embryos or to fertilise eggs with stored sperm. It can be assumed that the morphs are iteroparous batch breeders, which means that their embryo production is discontinuous. This is the case for *P. novaezealandiae s. str.* (Tutt 1997). For Onychophora in captivity this could only be substantiated by culturing a single female per box and by being fortunate enough to see the animals reproducing repeatedly.

On a premature hatchling from Boundary Stream an exuvium was seen, but no exuvia were noted on neonates. This might indicate that the embryos shed their cuticle shortly before birth. All morphs investigated are considered to be ovoviviparous, as is *P. novaezealandiae s. str*.

A differentiating characteristic for the morphs investigated might also be that it was possible to determine gender for some morph offspring after two months. Five months after birth, gender differentiation was possible for all morphs. Nevertheless the neonate genital openings were not as differentiated in pigmentation and colour, as the genital openings of adults. Possibly the orange papillae on younger juveniles are the precursors to the lateral anal papillae of males and to the area of orange papillae in females. Changes in pigmentation and brightness of the integument occurred over varying periods of time and might be characteristic for specific morphs. The deposition of urine drops at the nephropores during the investigation of juveniles is probably a sign of stress.

There are also some specific qualities that distinguish the observed morphs from *P. novaezealandiae s. str.* All observed animals were fairly robust and thus relatively easy to keep in captivity, a fact that contrasts to reports for *P. novaezealandiae s. str.* (Brosius 1998). Furthermore, early stages of embryos (Tutt 1997) from *P. novaezealandiae s. str.* and from Monckton look superficially identical, but later stages vary.

Another difference can be seen in the integument of the neonates. The coloration of the integument of newborn from Boundary Stream resembled that of newborn *P. novaezealandiae s. str.* (whitish or whitish purple), whereas the base colour of neonates from the other morphs was different (namely bluish grey). Rapid coloration of the integument might be useful in nature for the survival of the animals, because the glistening integument can probably be easily distinguished from the surroundings. The faster the juveniles are fully coloured, the better they may merge with the habitat and the harder it is for them to be seen by potential predators. However, it is always difficult to distinguish the juveniles and thus to relate them to the different stages. For future investigations it would be necessary to give the animals identity markings.

Many important criteria for differentiating morphs are lost on animals that have been preserved in ethanol (e.g. coloration of the integument of juveniles). Hence, the importance of observations on live animals in culture (in order to improve taxonomy) cannot be overemphasised. This work provides the first attempt to investigate morphological characters on sensitive, live onychophorans.

FUTURE DIRECTIONS

Because of their cryptic way of life under natural conditions, many behavioural aspects of Onychophora are still little understood. Much work remains to be done, especially since Onychophora are considered to be endangered through, for instance, the clearing of woodlands and over-collection (Barclay *et al.* 2000). Because of their special way of life, their complicated taxonomy, and a lack of information on their life cycle, only a few specialised conservation programmes have been developed for them. To secure the survival of onychophorans, investigations on taxonomy, ecology and behaviour of these animals should have high priority. In particular, ethological qualities might help to distinguish the taxa, and to determine the number of species in these areas of New Zealand.

New (1995) stresses that Onychophora co-occur with other cryptic invertebrate taxa, both in need of conservation. He especially points out that their ‘well-being mirrors that of many less conspicuous animals’. Thus, Onychophora may be regarded as ‘umbrella species’ and hence should be given high priority in nature conservation.

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REFERENCES

BARCLAY, S., ASH, J. E. & ROWELL, D. M. 2000. Environmental factors influencing the presence and abundance of a log-dwelling invertebrate, *Euperipatoides rowelli* (Onychophora: Peripatopsidae). *Zoological Journal of the Linnean Society* **250**: 425–436.

BROSIUS, H. 1998. Zur Problematik der Ovoviviparie unter besonderer Berücksichtigung der Onychophoren *Peripatoides novaezealandiae* und *Austroperipatus eridelos*. Diplomarbeit. Universität Hamburg, unpublished.

CROSBY, T. K., DUGDALE, J. S. & WATT, J. C. 1976. Recording specimen localities in New Zealand: an arbitrary system of areas and codes defined. *New Zealand Journal (Zoology)* **3**: 69 (+ separate map).

GLEESON, D. M. & RUHBERG, H. 2000. The onychophoran radiation in New Zealand: uncovered using a molecular approach. Abstracts, Congress Vol III. *Southern Connection Congress.*

HUTTON, F. W. 1876. On *Peripatus novae-zealandiae*. *The Annals and Magazine of Natural History* **18** (107): 361–369.

MANTON, S. M. 1938. Studies on the Onychophora. – VI. The life history of *Peripatopsis*. *Annals and Magazine of Natural History*. Ser. 11, **1**: 515–529.

NEW, T. R. 1995. Onychophora in invertebrate conservation: priorities, practices and prospects. *Zoological Journal of the Linnean Society* **114**: 77–89.

RUHBERG, H. 1981 *Peripatopsis moseleyi* (Onychophora) - Geburt. *Institut für den Wissenschaftlichen Film: Publikationen zu wissenschaftlichen Filmen. Sektion Biologie*, **14** (40). No. E 2666. Göttingen.

——— 1992. '*Peripatus'* – an approach towards a modern monograph. *Berichte des Naturwissenschaftlich-Medizinischen Vereins in Innsbruck*. Suppl. **10**: 441–458.

——— 1999. *Peripatoides* n. sp. – a new taxon of ovoviviparous onychophorans from Kapiti Island, New Zealand (Peripatopsidae, Onychophora). Abstract in: *Fragmenta Faunistica*, Supplement **42**: 50.

TUTT, K. 1997. The Life History and Reproductive Cycle of *Peripatoides novaezealandiae* (Onychophora: Peripatopsidae). Thesis submitted to the Victoria University of Wellington, unpublished.